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17 September 2004

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PATENT- OG VAREMÆRKESTYRELSEN

FIELD OF THE INVENTION

The present invention relates to a particle characterisation apparatus in which particles suspended in a liquid are passed through an orifice, in principle one by one, to enable the characterisation of the particles, for instance by Coulter counting.

BACKGROUND OF THE INVENTION

It is well-known that particles travelling through a small orifice can be characterised with respect to size, concentration and conductivity by the use of an electrical impedance technique, widely known as the Coulter sizing (see V. Kachel, "Electrical Resistance Pulse Sizing: Coulter Sizing", Flow Cytometry and Sorting, Second Edition, pp. 45-80, 1990 Wiley-Liss).

Counting and sizing of particles by the Coulter principle is an internationally respected method that is being used in most haematology-analysers and particle counting equipment. The method is based on measurable changes in the electrical impedance produced by non-conductive particles in an electrolyte. A small opening, called the "aperture" or "orifice", connects two electrically isolated chambers, where electrodes have been provided to contact the electrolyte. The orifice applies a restriction to the electrical path, whereby a sensing zone is established through which the particles are aspirated. In the sensing zone each particle will give rise to a displacement of the surrounding electrolyte, thus blocking part of the current-path and giving rise to a voltage pulse. By this method several thousand particles per second can be characterised with high precision.

It is also well-known that the peak amplitude of the voltage pulses generated by the particles are closely correlated to the size of the particles, and therefore it is desirable to be able to determine the peak amplitude of voltage pulses in a simple and reliable way and at a low cost.

SUMMARY OF THE INVENTION

According to the present invention these and other objects are fulfilled by a pulse height analyser for determination of the pulse height distribution of electronic pulses wherein the pulse height of each pulse is determined by recording of the passage of a set of voltage thresholds by the positive going edge of the pulse. The maximum threshold exceeded by the pulse characterises the peak amplitude of the pulse. Identification of the maximum exceeded threshold is input to a micro controller that is adapted to count the number of pulses within a pulse height category. A pulse height

category consists of pulses with a pulse height within a pulse height interval defined between respective threshold voltages.

Thus, the pulse height analyser may comprise a set of comparators with a common input that is provided with the electronic pulses.

Further, the pulse height analyser may comprise a set of latches wherein the inputs of the latches are connected to the outputs of respective comparators for recording passage of the corresponding threshold voltages by the rising edge of a pulse.

Identification of the set latched comparator outputs is input to the micro controller.

A priority encoder may be connected to the latch outputs for determination of a pulse height category consisting of pulses with a specific maximum exceeded threshold voltage. This minimises the number of inputs to the micro controller for provision of the category identification.

Further, the pulse height analyser may comprise a filter for provision of a substantially constant delay from pulse start to maximum pulse amplitude of the filtered pulse so that the time from pulse start to recording of the peak pulse amplitude is fixed whereby the electronic circuitry and especially the micro controller software handling the recorded measurement is simplified.

It is an important advantage of the present invention that the threshold voltages may be individually adjusted as desired. For example, it is not required that the threshold voltages are equidistant. If the possible sizes of the particles are known, it is possible to select a minimum of threshold voltages that are adjusted for optimum determination of the size distribution of the particles.

For example, in analysis of whole blood, it is desirable to count the number of three types of blood cells erythrocytes, leukocytes and thrombocytes. Their size, expressed as diameter, ranges from 2 μm for the smallest thrombocytes to 20 μm for the largest leukocytes.

Information on the content of leukocytes, their subpopulations and thrombocytes is an important tool for the physician in order to diagnose different diseases and monitor treatment. Furthermore, the concentration of haemoglobin, directly related to the number of erythrocytes, in the blood sample is also of great importance.

Thus, the number of erythrocytes, leukocytes and thrombocytes may be counted utilising the pulse height analyser of the present invention with threshold voltages that are selected and adjusted in accordance with the known sizes of the

erythrocytes, leukocytes and thrombocytes, e.g. by positioning threshold voltages in between corresponding mean values of the individual particle size distributions.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following the invention will be further described and illustrated with reference to the accompanying drawings in which:

Fig. 1 is a blocked diagram of a preferred embodiment of the present invention, and

Fig. 2 is a timing diagram illustrating the operation of the embodiment shown in Fig. 1.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Fig. 1 is a blocked diagram of a pulse height analyser 10 according to the present invention. The amplitude and frequency count of voltage pulses on the Signal_IN line is determined with the illustrated pulse height analyser 10. The operating principle of the analyser is described in the following with reference to the timing diagram of Fig. 2.

In Fig. 2, the voltage pulses 12 occurring on Signal_IN is illustrated as a square wave pulse. Typically, the pulses have varying pulse shapes with varying peak amplitudes, and varying pulse width.

The active filter 14 filters the input pulses 12, and the filtered output pulses 16 on the Signal_OUT line have a constant delay D1 between the peak amplitude 24 and the start of the rising edge of the pulses 16. D1 is independent of the peak amplitude of the pulses 12, 16. The ratio between the peak amplitude 24 of the filtered pulses 16 and the peak amplitude of respective input pulses 12 is substantially constant. As illustrated in Fig. 2, the pulse width of the output pulses is less than the pulse width of the input pulses. The determination of the pulse height analyser 10 is independent of the input pulse width.

The filtered output pulses 16 are provided to the N comparator & latches 18 with N thresholds. When the rising edge of the filtered pulse 16 passes a specific threshold THP (P = 1, 2, ... , N), e.g. threshold TH2 in Fig. 2, the corresponding latched output QP, e.g. Q2, is set. Thus, after D1 the latched outputs Q1, Q2, ... , QN that correspond to the thresholds TH1, TH2, ..., THN having been exceeded by the pulse in question are set. For example, in Fig. 2, the latched outputs Q1, Q2, and Q3 are set.

The N-Line to X-Line priority encoder 20 converts Q_N-Q_1 to a binary identifier A_X-A_1. In the example of Fig. 2, Q_N-Q_1 = 0...0111 is converted to binary A_X-A_1 = 0...0011. Thus the number of identifier bits is reduced. For example three identifier bits may identify up to 8 threshold voltages, four identifier bits may identify up to 16 threshold voltages, etc.

The Enable Output (EO) interrupts (INT) the micro controller 22 at the positive transition of EO generated when the rising edge of pulse 16 passes TH1.

The micro controller 22 contains a counter for each pulse height category so that the number of pulses occurring within each category may be counted. Preferably, consecutive threshold voltages define a pulse height category, i.e. output pulses 16 with peak amplitudes between thresholds TH1 and TH2 for which Q1 is set (Q_N...Q_2Q_1 = 0...0001) constitutes one pulse height category, and output pulses 16 with peak amplitudes between thresholds TH2 and TH3 for which Q1 and Q2 is set (Q_N...Q_2Q_1 = 0...0011) constitutes the next pulse height category, etc.

Upon interrupt, the micro controller 22 performs the following steps:

1) Wait D2 > D1 so that A_X-A_1 is stable (cf. Fig. 2),

2) READ A_X...$A_3A_2A_1$ (0...0011 in the example of Fig. 2),

3) Increment by one the counter in the micro controller 22 that counts the number of pulses 16 with the same peak amplitude recording, e.g. in the example of Fig. 2, the counter counting the number of pulses 16 with a peak amplitude 24 ranging from TH3 to TH4, and

4) Send CLR to the latches and the circuit is ready to receive the next pulse.

This procedure is repeated and executed in the measurement window. Measurement START and STOP signal to the micro controller 22 are not show in Fig. 1.

The priority encoder 20 may be realised with a 74HC148 8-Line to 3-Line priority encoder for N = 8 and X = 3.

Two 74HC148 8-Line to 3-Line Priority Encoder and three 2-input AND gates may be interconnected for provision of four binary identification bits, i.e. N = 16 and X = 4 as is well known in the art.

Likewise, as is also well-known in the art, 4 or 8 or 16 etc 74HC148 8-Line to 3-Line priority encoders and 3+4 = 7 or 7+5 = 12 or 12+6 = 18 etc 2-input AND gates may be interconnected for provision of five, six or seven, respectively, binary identification bits, i.e. N = 32, 64, or 128, and X= 5, 6, or 7, respectively.

In another embodiment of the present invention, the latches and priority encoder is embodied in a field programmable gate array (FPGA).

In yet another embodiment of the present invention, the circuitry illustrated in Fig. 1, exclusive the micro controller 22, is embodied in a hybrid application specific integrated circuit (ASIC) containing analogue and digital circuitry.

CLAIMS

1. A pulse height analyser for determination of the pulse height distribution of electronic pulses comprising

a filter for provision of a substantially constant delay from pulse start to maximum pulse amplitude of the filtered pulse,

a set of comparators with a common input that is connected to the filter output,

a set of latches wherein the inputs of the latches are connected to the outputs of respective comparators for recording passage of the corresponding threshold voltages by the rising edge of a pulse,

a priority encoder connected to the latch outputs for determination of a pulse height category consisting of pulses with a pulse height within a pulse height interval defined by respective threshold voltages, and

a micro controller that is adapted to count the number of pulses within each pulse height category.

2. An integrated circuit comprising a pulse height analyser according to claim 1.

3. A field programmable gate array comprising a pulse height analyser according to claim 1.

4. An application specific integrated circuit comprising a pulse height analyser according to claim 1.

5. A method for determination of the pulse height distribution of electronic pulses comprising the steps of:

defining a set of threshold pulse height values based on knowledge of possible pulse heights for optimum discrimination of pulse heights,

providing the pulses to a common input of a set of comparators, each of the comparators having a threshold input that is provided with a threshold voltage corresponding to a respective one of the threshold pulse height values,

recording passage of the corresponding threshold voltage by the rising edge of a pulse, and

counting the number of pulses within a pulse height interval defined by respective threshold voltages.

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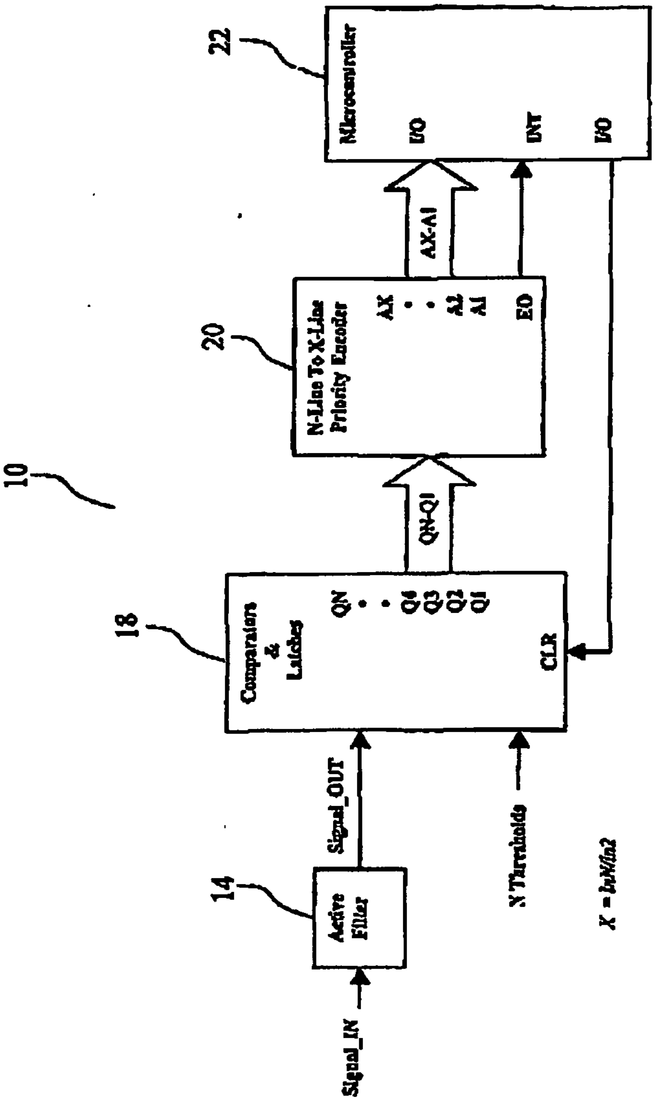
10
22
Microcontroller
I/O
INT
I/O
AX-A1
20
N-Line To X-Line Priority Encoder
AX
A2
A1
EO
QN-Q1
18
Comparators & Latches
QN
Q4
Q3
Q2
Q1
CLR
Signal_OUT
N Thresholds
14
Active Filter
Signal_IN
X = lnN/ln2

Fig. 1

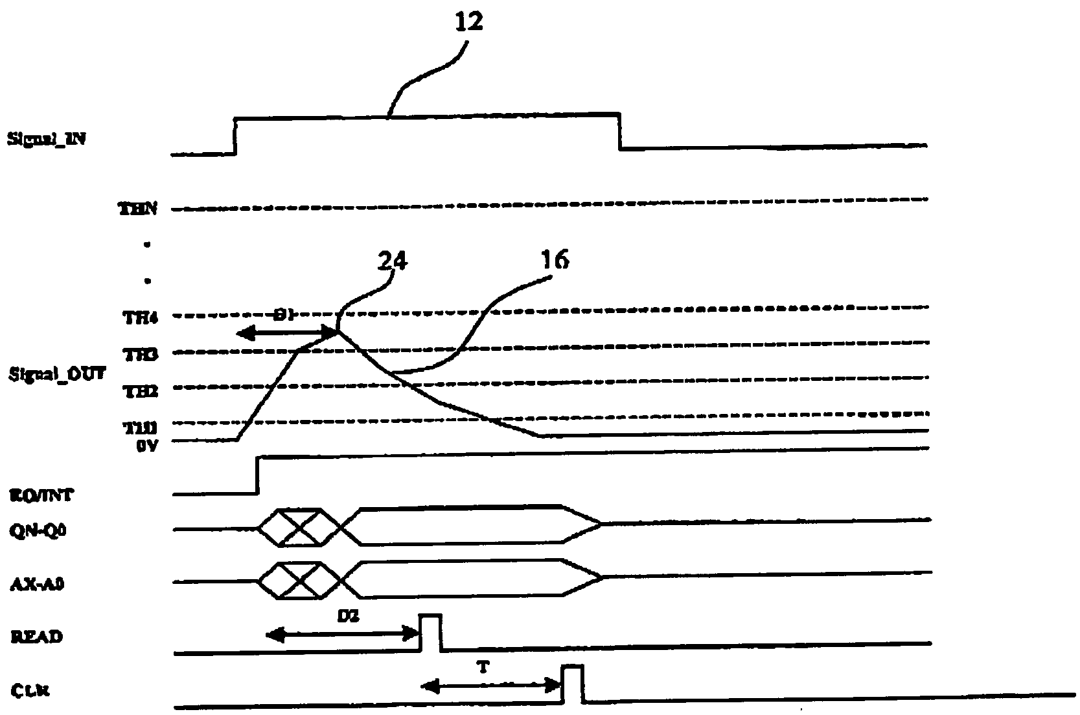
12
Signal_IN
THN
24
16
TH4
D1
TH3
Signal_OUT
TH2
TH1
0V
RQ/INT
QN-Q0
AX-A0
D2
READ
T
CLK

Fig. 2